5, 2000. This application also claims benefit of U.S. Provisional Application No. 60/179,486, filed February 1, 2000, of U.S. Provisional Application No. 60/155,107, filed September 22, 1999, of U.S. Provisional Application No. 60/152,233, filed on 13 September, 1999, and of U.S. Provisional Application No. 60/152,340, filed September 7, 1999. --

Please replace the paragraph beginning at line 21 of page 2 with the following amended paragraph:

-- The fact that an embryo and embryonic stem cells may be generated using the nucleus from an adult differentiated cell has exciting implications for the fields of organ, cell and tissue transplantation. For instance, embryonic stem cells generated from the nucleus of a cell taken from a patient in need of a transplant could be made, and induced to differentiate into the cell type required in the transplant. By using techniques evolving in the field of tissue engineering, tissues and organs could be designed from the cloned differentiated cells which could be used for transplantation. Because the cells and tissues used for the transplant would have the same nuclear genotype as the patient, the problems of transplant rejection and the dangers inherent in the use of immune-suppressive drugs would be avoided or decreased. Moreover, if necessary, the engineered cells and tissues could be readily modified with heterologous DNA, or modified such that deleterious genes are inactivated, so that the transplanted cells and tissues are genetically corrected. U.S. Application Serial No. 09/655,815, co-owned and filed concurrently with the present invention, discusses methods for genetically modifying both the donor nuclear DNA and the recipient mitochondrial DNA, and is herein incorporated by reference in its entirety. - -

Please replace the paragraph beginning at line 4 of page 5 with the following amended paragraph:

-- It has been proposed that the suppression of telomerase in differentiated cells may function to limit the capacity of somatic cells to expand in an uncontrolled manner clonally, as in cancer. But some tumor cell lines show a telomerase-negative immortality that has been designated the 'ALT' pathway. The inventors propose that this alternative pathway, like the acquisition of telomerase activity in tumorigenesis, is the reappearance of a germ line trait. The inventors propose that damaged telomeres are repaired in the germ line, not only through the addition of telomeric repeats by telomerase, but also through homologous strand invasion and extension by DNA polymerase. This is represented schematically in Figure 6, which depicts a damaged and a normal telomere, with the longer strand of the damaged telomere "invading" the normal telomere and being extended by DNA polymerase, with the complementary strand in the normal telomere serving as the template for DNA synthesis. --

A3

Please **delete** the figure at lines 12-17 of page 5.

Please replace the paragraph beginning at line 5 of page 6 with the following amended paragraph:

Not only does this theory have serious implications for the field of organ transplantation, it also calls into question the extent of genetic manipulations that can be performed on somatic cells that are to be used for nuclear transfer. For instance, a major advantage of nuclear transfer technology is that somatic cells may be more readily maintained in culture and transfected with transgenes than embryonic stem cells. This property facilitates the production of animals that produce therapeutic proteins, for instance, cows that express transgenes under control of mammary-specific promoters and produce

A4

therapeutic proteins in their milk. Likewise, if cells used for nuclear transfer are not permitted to undergo a series of genetic manipulations, either consecutively in culture, or consecutively through successive cloning, it will be virtually impossible to generate animals, cells, and tissues with multiple genetic manipulations. The ability to perform such complex genetic manipulations may be necessary, for example, before such cells are used for nuclear transfer and organ transplantation to correct genetic abnormalities in donor cells from patients having deleterious mutations. --

Please replace the paragraph beginning at line 6 of page 7 with the following amended paragraph:

-- Some researchers have suggested that telomerase activity may be cell-cycle dependent. For instance, in 1996, Dionne reported the down-regulation of telomerase activity in telomerase-competent cells during quiescent periods (G_0 phases) and hypothesized that telomerase activity may be cell-cycle dependent. See Dionne and Wellinger (1996) *Proc. Natl. Acad. Sci. USA* 93: 13902-13907. Similarly, Kruk et al. reported a higher level of telomerase in the early S phase when compared to other points in the cell cycle (*Biochem. Biophys. Res. Commun.* (1997) 233: 717-722). However, other researchers have reported conflicting results, and have alternatively suggested that telomerase activity correlates with growth rate, not cell cycle (Holt et al. (1996) *Mol. Cell. Biol.* 16(6): 2932-2939; see also Holt et al. (1997) *Proc. Natl. Acad. Sci. USA* 94: 10687-92; and Belair et al. (1997) *Proc. Natl. Acad. Sci. USA* 9: 13677-13682). Still others have proposed that telomerase activation is mediated by other cellular activation signals, as evidenced by the up-regulation of telomerase in B cells *in vitro* in response to CD40 antibody/antigen receptor binding and exposure to interleukin-4 (Weng et al. (1997) *Proc. Natl. Acad. Sci. USA* 94: 10827-32; see also Hiyama et al. (1995) *J. Immunol.* 155 (8): 3711-3715). But despite the rising interest in telomerase

and its purported role in the process of aging and cellular transformation, the regulation of telomerase activity remains poorly understood. See, e.g., Smaglik, "Turning to Telomerase: As Antisense Strategies Emerge, Basic Questions Persist," *The Scientist*, January 18, 1999, 13(2): 8.--

Please replace the paragraph beginning at line 19 of page 8 with the following amended paragraph:

-- The present invention is based on the surprising discovery, in light of the recent doubts about the genetic age of cloned mammals, that the process of nuclear transfer is capable of rejuvenating senescent or near-senescent cells, repairing tandemly repeating DNA sequence such as that in the telomeres, restoring youthful patterns of gene expression, such as increasing EPC-1 activity, and/or increasing cell life-span or cell proliferation capacity. The present invention therefore enables what would not have been deemed possible in light of the recent concerns about nuclear transfer; namely, that cells that are at or near senescence, e.g., those grown in culture until they are near senescence, or obtained from humans or animals having age-related defects or conditions, may still be used to generate cloned cells, tissues, and animals having telomeres that are at least comparable in length, or longer than, the telomeres of age-matched control cells, tissues, and animals. Also, these cells possess patterns of gene expression of young cells, such as increased EPC-1 activity relative to that of donor cells. Moreover, the present invention establishes, in contrast to what has recently been suggested, that generating clones of clones, i.e., "re-cloning," is entirely feasible, and may be repeated theoretically indefinitely, thereby resulting in the generation of "hyper-young" cells, tissues, organs and animals. –

Please replace the paragraph beginning at line 11 of page 9 with the following amended paragraph:

-- Telomere shortening, schematically depicted in Figure 7, is currently believed to lead to chromosome ends that, to a cell, are indistinguishable from double strand breaks and signal DNA damage, resulting in cell cycle arrest at a DNA damage checkpoint (W.E. Wright & J. S. Shay, 2000, Nat. Med. 6(8) 849-851). –

Please **delete** the figure at lines 14-17 of page 9.

Please replace the paragraph beginning at line 18 of page 9 with the following amended paragraph:

--Telomeres may, however, contain an increasing amount of degenerate or non-telomeric repeat DNA progressing from the centromere, as shown in Figure 8. --

Please **delete** the figure at from line 20 of page 9 to line 4 of page 10.

Please replace the paragraph beginning at line 5 of page 10 with the following amended paragraph:

-- The appearance of these non-telomeric repeat sequences causes a temporary DNA damage checkpoint. Following repair, such as through exonuclease activity, the cell can re-enter the cell cycle. As shown in Figure 9, the progression of a mortal cell to terminal senescence is accompanied by progressive degradation of the telomeric DNA; and subsequent nuclear transfer of senescent chromosomes, from which telomeric DNA has been removed, causes the synthesis of an extended array of uniform telomeric repeat sequences (Figure 10) that do not always appear in nature. Cells and/or animals containing chromosomes with such extended and uniform telomeric repeat sequences will be

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rejuvenated, and have the unique characteristic of being hyper-young, as a mass population of cells having fewer cells in DNA damage checkpoint at any one period of time. --

Please **delete** the figure at lines 10-12 of page 10.

Please replace the paragraph beginning at line 3 of page 11 with the following amended paragraph:

-- In this regard, researchers at Geron Corporation and the Roslin Institute have recently collaborated to combine Geron's cloned telomerase gene (hTERT) with nuclear transfer in order to resolve telomere shortening in clones. See, e.g., Business Wire, May 26, 1999. This announcement preceded the May 27th Nature report by researchers at Roslin Institute that two other sheep cloned by nuclear transfer (after Dolly) also exhibit shorter telomeres than age-matched controls. Researchers at the University of Massachusetts involved in cloning cattle also believed that transfecting donor cells with an exogenous telomerase gene might be beneficial for the life-span of cloned animals, despite their observation that nuclear transfer seemed to rejuvenate senescent donor cells. See ABC News (Reuters), Daily News, May 22, 1998.--

Q10

Please insert the following paragraphs describing **Figures 6-10** after line 8 on page 18, immediately before the caption "**Detailed Description of the Invention.**"

-- **Figure 6**. Schematic representation of proposed model for the erosion of telomeric $(T_2AG_3)_n$ repeats from the terminus of chromosomal DNA. Fig. 6(a) depicts telomeric DNA as consisting of an "imperfect" internal region of $(T_2AG_3)_n$ repeats (shaded) in which non-T_2AG_3 sequences (unshaded) are interspersed, and a terminal region of uninterrupted $(T_2AG_3)_n$ repeats. Fig. 6(b) depicts DNA of a similar telomere from which the terminal $(T_2AG_3)_n$ repeats have been removed, leaving at the terminus a non-T_2AG_3 tract that cannot

Q11

bind TRF2. It is proposed that failure of TRF2 to bind to the terminal chromosomal DNA signals an increase in the cellular activities of p53 and p21, which triggers cellular senescence.

Figure 7 schematically depicts the end of a chromosome that terminates in telomeric repeat DNA sequences (top), and the terminus of the same chromosome after loss of the telomeric DNA (bottom), as occurs when a cell becomes senescent.

Figure 8 schematically depicts the presence of an increasing amount of degenerate or non-telomeric repeat DNA sequences between telomeric repeat DNA sequences, progressing from the telomere toward the centromere, and illustrates how degradation of telomeric DNA sequences from the terminus of such a chromosome (top) creates a terminus ending in a non-telomeric sequence that the cell detects as damaged DNA (bottom).

Figure 9 schematically depicts a chromosome that terminates in a non-telomeric DNA sequence (top), which sends the cell into DNA damage checkpoint arrest; the same chromosome after damage repair has removed the terminal non-telomeric DNA (middle); and the end of the chromosome after all telomeric DNA has been lost (bottom).

Figure 10 schematically depicts the end of a chromosome of a senescent cell, showing loss of all telomeric DNA (top); and the end of the same chromosome after an extended array of uniform telomeric repeat sequences has been synthesized following nuclear transfer. --

Please replace the paragraph beginning at line 1 of page 21 with the following amended paragraph:

-- The differentiated cells, teratomas, inner cell masses, embryonic disc cells, and embryonic stem cells isolated according to the invention will have telomeres that are at least

as long, if not longer, than those of the donor normal somatic cell, and are also an aspect of the invention. These cells can also possess increased EPC-1 activity. A method whereby the differentiated cells or tissues, teratoma cells, inner mass cells, blastocyst cells or embryonic cells are then used as subsequent nuclear donors is also envisioned. Such a method is particular suitable for isolating normal somatic cells, teratomas, ES cells, etc. having multiple transgenes or genetic alterations, and may be repeated indefinitely until the desired number of genetic changes have been accomplished. --

Please replace the paragraph beginning at line 10 of page 21 with the following amended paragraph:

--The normal somatic cell used for the methods of the invention may be any cell type. Suitable cells include by way of example immune cells such as B cells, T cells, dendritic cells, skin cells such as keratinocytes, epithelial cells, chondrocytes, cumulus cells, neural cells, cardiac cells, esophageal cells, dermal fibroblasts, cells of various organs including the liver, stomach, intestines, lung, pancreas, cornea, skin, gallbladder, ovary, testes, other reproduction organs, kidneys, etc. In general, the most appropriate cells are easily propagated in tissue culture and can be easily transfected. Fibroblasts are preferable cell types for transfecting heterologous DNA and performing nuclear transfer.--

Please replace the paragraph beginning at line 4 of page 25 with the following amended paragraph:

-- These cells and tissues, which optionally may be transgenic, may be used for cell, tissue and organ transplantation, e.g., treatment of burns, hair transplantation, cancer, chronic pain, diabetes, dwarfism, epilepsy, heart disease such as myocardial infarction, hemophilia, infertility, kidney disease, liver disease, osteoarthritis, osteoporosis, stroke, affective disorders, Alzheimer's disease, enzymatic defects, Huntington's disease,

a14 hypocholesterolemia, hypoparathyroidism, immunodeficiencies, Lou Gehrig's disease, macular degeneration, multiple sclerosis, muscular dystrophy, Parkinson's disease, rheumatoid arthritis, spinal cord injuries and other trauma. --

Please replace the paragraph beginning at line 8 of page 29 with the following amended paragraph:

-- For instance, Xu et al. demonstrated that re-expression of the retinoblastoma protein in tumor cells induces senescence and inhibits telomerase activity (Oncogene (1997) 15: 2589-2596). A recent report also suggests that a gene on chromosome 3 may be involved in transcriptional repression of hTERT, the catalytic subunit of telomerase. See Horikawa et a15 al. (1998) Mol. Carcinog. 22(2): 65-72. Several proteins have also been identified that interact directly with telomerase, such as p23/hsp90 (molecular chaperones) and TEP1 (telomerase associated protein 1). See Id. Researchers at Lawrence Berkeley National Laboratory have purportedly cloned two additional human telomere-associated proteins (Tin 1 and Tin 2). See Federal Technology Report, December 30, 1999, Partnership Digest, Technology Watch, p. 9. Thus, the regulatory mechanism identified by the present methods could operate by binding to or inhibiting the expression of a telomerase binding protein or a telomerase repressor, consequently increasing telomerase activity, but could also regulate telomerase activity by upregulating gene expression or enhancing protein stability. --

Please replace the paragraph beginning at line 17 of page 37 with the following amended paragraph:

-- In discussions about cloning, it is commonly asked whether the animals generated a16 by nuclear transfer are the result of the use of some rare cell rather than the majority of the cells in the culture. Mass cultures have multiple lineage's with various maximum achievable

cell life-spans (43). Indeed, the late passage cells used in the present study represent cells that originally had the greatest life-span. If there were a subset of young cells with twenty or more population doublings remaining in the late passage culture, they would have out-proliferated the culture as is seen in mouse cell culture, where spontaneous immortalization is common. In anticipation of this objection, we plated the donor cells at clonal densities and scored the proliferative life-span of every cell. Three-hundred and thirty-nine of the 347 cells (98%) underwent fewer than three PDs, whereas 347/347 (100%) underwent four or fewer PDs. Furthermore, the cells were grown in high serum (15%) concentrations, and young cells would have been rapidly proliferating and easily observed in the dish. The probability of a young cell in our sample is therefore less than 1/347. Seven animals (6 term animals and 1 fetus) were nevertheless cloned from the population of senescent fetal cells. It is therefore highly improbable that we, by chance, cloned the animals from undetectable young cells ($P<0.001$, Chi-square).--

Please replace the paragraph beginning at line 10 of page 42 with the following amended paragraph:

-- Minor senescence problems have been repaired by 3'→5' exonuclease that then again exposes T_2AG_3, which restores binding to TRF-2. However, at some point the damage is so substantial it triggers what is known as terminal cell senescence. --

Please replace the paragraph beginning at line 13 of page 42 with the following amended paragraph:

-- Wilmut argued that cloning from a senescent cell may lead to problems in animals because telomere length reflects the shortened telomere of the somatic cell donor nucleus. However, our results suggest the exact reverse. Rather, growing a cell to senesence or near-senescence, or checkpoint arrested, allowing the cell to lose T_2AG_3, removing minor damage

along the way by 3'→5' exonuclease may afford an opportunity to then transfer that gene into an enucleated oocyte or other embryonic cell and with a subsequent burst of telomerase activity to rebuild a tract of pure T_2AG_3 (longer than normally present). These cells will possess longer life-spans, but also, because of the purity of T_2AG_3 would rarely have cells in temporary cell cycle arrest. This would result in higher than normal mitotic cell index and overall a "younger than young" pattern of gene expansion. --

Please replace the paragraph beginning at line 1 of page 43 with the following amended paragraph:

-- To more thoroughly investigate this, experiments are being conducted using cultures from age-matched mammals and cloned samples, cloned from young and senescent cells and those with or without shortened telomeres. These cells are grown to senescence and frozen back every 15 PD. These cells will be compared with respect to markers of cell senescence. Gene expression will be compared in these cells by known methods, e.g. northern blots, or by labeling with suitable probes. --

Please replace the paragraph beginning at line 15 of page 46 with the following amended paragraph:

-- Our results suggest that the artificial removal of telomeres through senescence, and then the rapid re-synthesis of accurate TTAGGG following nuclear transfer, may lead to cells and animals that have the ability to proliferate in a younger state longer than a normal cell. There is no reason for evolution to select for cells or animals that would live longer than they need to in order to reproduce. So there is no reason for the germ line to give the somatic cells more uniform TTAGGG repeat sequences than they need. The technique of growing cells to senescence would effectively strip away the good and the bad telomeric sequences, and then nuclear transfer would give the cells a better longevity potential than they ever had

normally. This would be the case even if the cells had telomere lengths comparable to those of normal cells. This would lead to cells that had a higher mitotic index for a longer period of time, and therefore to animals that aged better and lived longer. --

Please replace the paragraph beginning at line ~~15~~ 3 of page ~~46~~ 47 with the following amended paragraph:

-- Therefore, the uses of the subject nuclear transfer method with telomere extension, or even without telomere extension, may result in the re-synthesis of new uniform TTAGGG repeats in the telomeres. While not being bound by their hypothesis, the inventors believe that this may occur via the up-regulation of telomerase and EPC-1, alone or in association with other genes such as growth arrest sequences (*gas* genes), collagenase, tPA, and others. This should result in longer lived and healthier animals, and cells for human therapy that are "hyper-youthful." This is the first demonstration of hyper-youthful cells, that is, a population of cells and tissues with an overall phenotype that is, even more young than a normal mixed population of young cells, i.e., having a pattern of gene expression and mitotic index more youthful than normal youthful cells. The use of telomerase merely extended telomeres and the life-span of cells. However, to the inventors' knowledge, all of the published reports showed no evidence that cells could be obtained wherein the overall phenotype of such cells is younger or hyper-young. Indeed, many researchers report that old, but not yet senescent cells that have slowed down continue to divide slowly, but indefinitely, with telomerase. --

Please replace the paragraph beginning at line 22 of page 47 with the following amended paragraph:

-- Animals cloned from senescent cells using this technology would be predicted to have unique properties. Such animals would be predicted to have an increased immune